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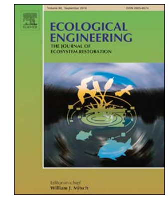

Research Paper

Toxicity assessment of *Bacopa monnieri* L. grown in biochar amended extremely acidic coal mine spoils

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ABSTRACT

The addition of biochar in acidic mine spoils contaminated areas has been suggested for buffering and improving the fertility as well as the reduction in the metal bioavailability. This study evaluated the effect of biochar on reducing the heavy metal content in the *Bacopa monnieri* L. grown in the biochar amended acidic mine spoil and associated toxicological risks. The mine spoil (overburden) was treated with a biochar made from the fast pyrolysis of waste of *Cymbopogon flexuosus* at 450 °C at application rates of 15% and 20%. Plant analysis (biomass, chlorophyll, protein and lipid peroxidises), metabolite content and metal levels (Al, Cd, Cr, Cu, Fe and Pb) in the *Bacopa monnieri* L. herb. Hazardous quotient and acute oral toxicity of plant and its extract cultivated was carried out. The results showed that biochar amendment significantly improved biomass of the plant as well as reduced the metal content in plant tissues. The concentrations of Al, Cd, Cr, Cu, Fe and Pb in the plant tissues were within the international permissible limits. The risk assessment for bacopa consumption showed that the hazard quotients values were lower than the threshold level for herb (< 1). Application of biochar further reduced hazard quotients values the indicating safe to the consumers. In vivo toxicity also suggests that no toxic effect of bacopa extract on the albino mice. It is concluded that the application of biochar in acidic mine spoils can be not only useful for the plant growth, but also for reduction in the metal toxicity of *Bacopa monnieri* L., if consumed.

1. Introduction

Extremely acidic coil mine spoils pose potential hazards to plant and human being due to metal toxicity. Under the natural conditions, acidification and heavy metal toxicity of these mine spoils act synergistically which leads the acceleration in the toxic metals leaching rate at extremely phytotoxic levels (Anawar 2015; Liao et al., 2007). The liming and sorptive properties of biochar make it promising for remediation of acid and metal contaminated soils such as mine spoils (Rodríguez-Vila et al., 2015). The incorporation of biochar in soil contaminated with overburdens (mine spoils) can serve dual purpose i. Iimprove soil fertility and ii. Mitigate the risk of heavy metal contamination in various environmental compartments (Hossain et al., 2015).

The reduction in the metal bioavailability is well documented in the literature not only in the acidic mine spoils (Ippolito et al., 2017), but also in other metal contaminated soils (Al-Wabel et al., 2015; Rizwan et al., 2016). Numerous pot studies indicate that biochar can significantly reduce bioaccumulation of toxic metals in vegetable (Beesley et al., 2013; Khan et al., 2015; Waqas et al., 2014; Waqas et al., 2015), rice (Khan et al., 2014) and other plant species (Houben et al., 2013a,b; Liu et al., 2014). Most of the studies reported on the biochar amendments in mine spoils were as an aided phyto-stabilization with phytoremediation. Their major emphasis was on improvements in the soil fertility after applying the biochar. Data on the metal translocation in the plant grown in the biochar amended highly acidic mine spoil is limited (Rodríguez-Vila et al., 2016; Rodríguez-Vila et al., 2017) and scarce for coal mines. This is the first time report of examining toxicity of plant grown in biochar amended highly acidic coal mine spoil.

This investigation is in continuation with our previous study, in which we reported the soil biological activities after biochar amendments in acidic mine spoils (Jain et al., 2016). Here, we examined the response of biochar for reducing the phytotoxicity of Al, Cd, Cr, Cu, Fe and Pb in *Bacopa monnier* L. cultivated in contaminated mine soil. The

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hypotheses for the study are 1. What is the effect of BC on the metal accumulation on the plant? 2. Whether the *Bacopa monnier* L grown in the area of mine spoil could have any toxic effect on human health after consumption?

To achieve theses aims, bacopa plants were selected for this study firstly, it could be easily grown in the marshy land and good phytoremediator (Mishra et al., 2016; Singh et al., 2006). Secondly, it is wildly cultivated and one of the most important plants in Ayurveda (Mishra et al., 2016). Bacopa has been used in India for 5000 years as an alternative medicine (Muszyńska et al., 2016). Metal accumulation and translocation in plant was evaluation in the biochar amended overburden and compared that with overburden. The toxicity of powder and aqueous extract from the bacopa was assessed by Hazardous quotient and acute oral toxicity, respectively. Hematological and biochemical parameters were also done to assess the possible toxic effect of bacopa extract in mice.

2. Material and method

2.1. Sample collection

Overburden sample was collected from the coal fields of Jaintia Hills (25°100 and 25°280 N, 92°080 and 92°340 E), Meghalaya, India. The sample was ground to a mesh size of 72 BS(0.211 mm), preserved in desiccators, and used in all subsequent analyses. Biochar were prepared from *Cymbopogon flexuosus* (lemongrass) following the procedure described in Section 2.2.

2.2. Biochar preparation

The biomass samples were sieved for particle size of 0.210 mm and pyrolyzed in a programmed temperature fixed bed reactor (capacity 300 g ± 60, tube dia 50 mm, sample bed upto 300 mm) at 450°C, for 1 h. The pyrolysis was conducted in nitrogen gas atmosphere. The heating rate of furnace was 10°C. Biochar physicochemical properties were determined through proximate, ultimate and functional group chemistry analyses. Proximate analysis was done by ASTM method D3172. RSD (5) for replicates of moisture, volatile matter and ash contents were 0.36, 0.17 and 0.03, respectively.

2.3. Pot experiments and treatments

Pot trials were set up in seven treatments two overburden and soil and at two biochar rates with soil blank as a control. *B.monnieri* in each pot was grown for a cropping period. Nursery of *B. monnieri* was taken from the Central Institute of Aromatic and Medicinal Plants (CIMAP) farm, Lucknow, India. Triplicates of each treatment were taken for the study. The biochars were applied to the soils in a pot experiment as detailed by (Lu et al., 2014). The amendment mixture was made of using different proportion of soil and overburden according to the treatment details given in Table 1.

Table 1
Ratio of soil and OB and application rate of biochar in different treatment.

Treatments	Name	Biochar (%)	Soil (Kg)	OB (Kg)
T1	Soil (blank)	–	2.0	–
T2	Soil: OB + BC (75:25 + 15%)	15	1.5	0.5
T3	Soil: OB (75:25)	–	1.5	0.5
T4	Soil: OB + BC (75:25 +20%)	20	1.5	0.5
T5	Soil: OB + BC (50:50 + 15%)	15	1.0	1.0
T6	Soil: OB (50:50)	0	1.0	1.0
T7	Soil: OB + BC (50:50 + 20%)	20	1.0	1.0

OB: Overburden; BC: Biochar.

Table 2
Physico-chemical properties of Overburden, biochar and soil (Jain et al., 2016).

	C	H	N	S	IS	TP	AvP	OC	N_{min}	pH	Na[a]	K[a]	Ca[a]	Co[a]	Cr[a]	Cu[a]	Fe[a]	Mg[a]	Mn[a]	Ni[a]	Pb[a]	Zn[a]
	(%)					(mg/kg)		(%)														
Overburden	22.3	2.6	1.1	3.1	0.25	114	2.3	0.31	84	3.6	280	83	375	7	17	4	141	1354	92	7	13	37
	(1.9)	(0.2)	(0.1)	(0.4)	(0.02)	(8.8)	(0.2)	(.02)	(7.0)	(0.4)	(16.7)	(6.5)	(18.5)	(0.92)	(1.5)	(0.52)	(10.67)	(41.32)	(7.9)	(0.98)	(1.4)	(2.8)
Biochar	51	3.6	1.2	ND	BDL	3998	58.6	9.71	112	10.1	180	542	52	ND	ND	ND	17	824	80	6	ND	21
	(4)	(0.3)	(0.1)			(51.0)	(5.0)	(1.0)	(7.2)	(1.2)	(9.3)	(23.6)	(3.9)				(1.9)	(29.3)	(6.9)	(0.57)		(2.1)
Soil	0.73	0.2	1.6	0.1	0.09	1030	9.8	0.43	224	7.2	107	17	1094	0.19	0.61	0.40	391	1343	52	4.9	0.26	13.5
	(.05)	(.01)	(0.1)	(.001)	(.006)	(32.0)	(1.0)	(.003)	(12.3)	(0.91)	(6.9)	(1.9)	(35.6)	(0.02)	(0.003)	(0.03)	(20.1)	(40.1)	(3.9)	(0.39)	(.02)	(1.8)

Errors are given in parenthesis.
[a] Concentration in ppm.